In Vitro study on phytonutrients potential and human health promoting properties of fatty acids isolated from seeds of *Vicia faba* collected from East Medinipur, West Bengal

Ph.D. SYNOPSIS

SUBMITTED TO THE
Y.B.N. UNIVERSITY, RANCHI (JHARKHAND)

FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
FOOD AND NUTRITION
(DEC 2022)

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Established by the Act of Government of Jharkhand Act 15, 2017
Gazette Notification No. 505, Dated 17th July 2017
As per Section 2(f) of UGC Act. 1956

Synopsis Title: *In Vitro* study on phytonutrients potential and human health promoting properties of fatty acids isolated from seeds of *Vicia faba* collected from East Medinipur, West Bengal

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ABSTRACT

This study aims to investigate the *in vitro* antimicrobial, antioxidant, enzyme inhibitory, anti-diabetic, and overall health-promoting properties of fatty acids extracted from *Vicia faba* seeds. A comprehensive literature review will be conducted to establish the scientific rationale for this research and identify knowledge gaps. Fatty acids will be extracted from *Vicia faba* seeds using a standardized protocol. The extracted fatty acids will then be subjected to various *in vitro* assays to assess their antimicrobial activity against a panel of pathogenic bacteria and fungi. Additionally, their antioxidant potential will be evaluated using standard assays. Enzyme inhibitory activities, such as α-amylase and α-glucosidase inhibition, will be determined to explore their potential for managing diabetes. Future studies will involve *in vivo* models to validate the *in vitro* findings and explore the potential therapeutic applications of these fatty acids.

Fatty acids are vital nutrients that contribute to overall health. By understanding the different types of fatty acids and their respective health benefits, individuals can make informed choices about their diet to support optimal well-being. It is essential to consult with a healthcare professional for personalized dietary advice.

The expected outcomes of this study include a detailed characterization of the fatty acid profile of *Vicia faba* seeds and a comprehensive evaluation of their biological activities. It is anticipated that this research will provide scientific evidence supporting the traditional

use of *Vicia faba* and contribute to the development of novel natural products with potential applications in the pharmaceutical, food, and cosmetic industries. This research aims to investigate and elucidate the potential antimicrobial, antioxidant, enzyme inhibitory, anti-diabetic, and overall health-promoting properties of fatty acids extracted from *Vicia faba* seeds.

Key-words: *Vicia faba*, Fatty acids, Enzymes, Microorganisms, Antioxidant activity, Diabetes

INTRODUCTION

The escalating global burden of chronic diseases, such as diabetes, cardiovascular disorders, and infectious diseases, underscores the urgent need for innovative therapeutic strategies. While synthetic drugs have revolutionized healthcare, the emergence of antimicrobial resistance and the adverse side effects associated with many conventional medications have prompted a renewed interest in exploring natural alternatives. Plants, with their rich repository of bioactive compounds, have emerged as promising sources for drug discovery and development.

Vicia faba, commonly known as broad bean or faba bean, is a legume widely cultivated for its edible seeds. Beyond its nutritional value, this plant has been traditionally used in folk medicine for the management of various ailments. However, the scientific basis for these traditional claims remains largely unexplored. Emerging evidence suggests that plant-derived fatty acids possess a wide spectrum of pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory activities.

Given the growing interest in functional foods and nutraceuticals, the exploration of *Vicia faba* seeds as a potential source of bioactive fatty acids is warranted. This research aims to investigate the in vitro antimicrobial, antioxidant, enzyme inhibitory, anti-diabetic, and overall health-promoting properties of fatty acids isolated from *Vicia faba* seeds. By characterizing the fatty acid profile and evaluating their biological activities, this study seeks to provide scientific evidence for the traditional use of *Vicia faba* and contribute to the development of novel natural products with potential therapeutic applications.

A comprehensive understanding of the bioactive constituents in *Vicia faba* seeds is essential for unlocking their full therapeutic potential. While previous studies have focused on other phytochemicals such as flavonoids and saponins, the role of fatty acids in the pharmacological properties of this legume remains largely unexplored. This research gap provides a compelling rationale for investigating the fatty acid composition and bioactivities of *Vicia faba* seed oil.

By elucidating the antimicrobial, antioxidant, and enzyme inhibitory properties of *Vicia faba* seed fatty acids, this study aims to contribute to the development of novel strategies for combating infectious diseases and oxidative stress-related disorders. Additionally, the evaluation of the anti-diabetic potential of these fatty acids offers a promising avenue for addressing the global diabetes epidemic. Ultimately, this research seeks to provide a scientific foundation for the utilization of *Vicia faba* as a functional food ingredient or as a source of natural drug leads.

The specific objectives of this research are:

1) To isolate various types of fatty acids from the seeds of *Vicia faba*.
2) To evaluate the antimicrobial activity of the isolated fatty acids against a variety of bacteria.
3) To determine the antioxidant capacity of the fatty acids by evaluating DPPH, ABTS, and FC Radical Scavenging & ROS Scavenging activity.
4) To evaluate the effects of fatty acids on the activity of enzymes such as alpha-amylase, alpha-glucosidase, and protease and the role of inhibition of these enzymes in the management of diabetes.
5) To study the potential health-promoting effects of fatty acids, such as reducing the risk of heart disease, reducing inflammation, and strengthening the immune system.

LITERATURE REVIEW

A comprehensive review of existing literature on the pharmacological properties of *Vicia faba* was undertaken. Previous studies have explored various aspects of this legume, including its nutritional composition, antinutritional factors, and some preliminary

pharmacological activities. For instance, research has demonstrated the presence of bioactive compounds such as flavonoids and saponins in *Vicia faba* seeds, which have been associated with antioxidant and anti-inflammatory effects (Kumar et al., 2010). However, the specific focus on fatty acids and their potential health benefits from this particular legume remains limited.

While there is substantial research on the antimicrobial, antioxidant, and anti-diabetic properties of fatty acids derived from different sources, the exploration of these properties in the context of *Vicia faba* seed oil is scarce. Although studies have indicated the presence of fatty acids in *Vicia faba* seeds, their detailed characterization and biological evaluation are lacking. Furthermore, the synergistic effects of different fatty acid components in relation to the aforementioned properties have not been comprehensively investigated.

Extensive research has been conducted on the nutritional and pharmacological properties of *Vicia faba* (Broad bean). Previous studies have primarily focused on the protein content and antinutritional factors of this legume (Kumar et al., 2010). However, the exploration of its lipid profile and associated bioactivities remains relatively understudied. While some investigations have reported the presence of fatty acids in *Vicia faba* seeds, a comprehensive analysis of their composition and potential health benefits is lacking (Smith et al., 2015).

The literature is replete with studies highlighting the antimicrobial, antioxidant, and anti-diabetic properties of fatty acids derived from various sources, including fish oil, flaxseed oil, and olive oil (Brown et al., 2012; Johnson et al., 2018). These studies have demonstrated the potential of fatty acids as promising therapeutic agents for a wide range of diseases. Nevertheless, the specific contribution of *Vicia faba* seed fatty acids to these beneficial effects remains largely unexplored.

A considerable gap exists in our understanding of the relationship between the fatty acid composition of *Vicia faba* seeds and their associated bioactivities. Furthermore, the synergistic effects of different fatty acid classes in exerting these properties have not been

thoroughly investigated. This study aims to address these knowledge gaps by conducting a comprehensive analysis of the fatty acid profile of *Vicia faba* seeds and evaluating their antimicrobial, antioxidant, enzyme inhibitory, and anti-diabetic properties. By filling this research void, this study will contribute significantly to the existing body of knowledge on the pharmacological potential of this underutilized legume.

This study aims to bridge this knowledge gap by conducting a detailed in vitro investigation of the antimicrobial, antioxidant, enzyme inhibitory, and anti-diabetic properties of fatty acids isolated from *Vicia faba* seeds. By characterizing the fatty acid profile and evaluating their biological activities, this research will contribute to the understanding of the potential therapeutic applications of this underutilized plant resource.

RESEARCH METHODOLOGY

Research Location and Design

This study will be conducted at the Department of Food and Nutrition, College of Science, East Medinipur, West Bengal, India. A laboratory-based experimental design will be employed to investigate the antimicrobial, antioxidant, enzyme inhibitory, anti-diabetic, and health-promoting properties of fatty acids isolated from *Vicia faba* seeds.

MATERIALS AND METHODS

Sample Collection: fresh Vicia faba seeds will procure from local farmers in East Medinipur district during the harvesting season. The seeds will be thoroughly cleaned, dried, and stored at room temperature until further use.

Extraction of Fatty Acids: Fatty acids will be extracted from *Vicia faba* seeds using a modified Bligh and Dyer method. The extracted lipids will be subjected to transesterification to obtain fatty acid methyl esters (FAMEs), which will be analyzed using gas chromatography-mass spectrometry (GC-MS) to determine the fatty acid profile.

1. Isolation of Fatty Acids from *Vicia faba* Seeds

Materials:

- *Vicia faba* seeds
- Petroleum ether, Methanol, Chloroform
- Soxhlet apparatus
- Rotary evaporator
- Gas Chromatography-Mass Spectrometry (GC-MS)

Methods:

- The seeds of *Vicia faba* will be cleaned, dried, and ground into a fine powder.
- The fatty acids will be extracted using a Soxhlet apparatus with petroleum ether as the solvent. The extraction process will continue until the solvent becomes colorless.
- The extracted solvent will be evaporated using a rotary evaporator under reduced pressure to obtain crude fatty acids.
- The fatty acids will then undergo saponification to remove neutral lipids.
- The methyl esters of the fatty acids will be prepared using methanol and sulfuric acid.
- The fatty acid methyl esters (FAMEs) will be analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) for identification and quantification.

2. Evaluation of Antimicrobial Activity

Materials:

- Isolated fatty acids
- Bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*
- Mueller-Hinton Agar (MHA)
- Dimethyl sulfoxide (DMSO)
- Sterile discs

Methods:

- The antimicrobial activity of the isolated fatty acids will be evaluated using the disc diffusion method.
- Mueller-Hinton Agar plates will be prepared, and the bacterial strains will be inoculated onto the plates.
- Sterile discs impregnated with different concentrations of the fatty acids (dissolved in DMSO) will be placed on the inoculated plates.
- The plates will be incubated at 37°C for 24 hours.
- The zones of inhibition will be measured and recorded to assess the antimicrobial efficacy of the fatty acids.

3. Determination of Antioxidant Capacity

Materials:

- Isolated fatty acids
- 2,2-Diphenyl-1-picrylhydrazyl (DPPH)
- 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)
- Ferric chloride (FeCl3)
- Sodium carbonate (Na2CO3)
- Folin-Ciocalteu reagent (FC)
- Hydrogen peroxide (H2O2)
- Phosphate buffer

Methods:

DPPH Radical Scavenging Activity:

- The DPPH radical scavenging activity of the fatty acids will be determined by mixing the fatty acid solution with DPPH in methanol.
- The mixture will be incubated in the dark for 30 minutes, and the absorbance will be measured at 517 nm using a spectrophotometer.
- The percentage of radical scavenging activity will be calculated.

ABTS Radical Scavenging Activity:

- The ABTS radical cation will be generated by reacting ABTS with potassium persulfate.
- The fatty acids will be added to the ABTS solution, and the absorbance will be measured at 734 nm after incubation for 30 minutes.
- The percentage of inhibition will be calculated to determine the antioxidant capacity.

Folin-Ciocalteu (FC) Reducing Power Assay:

- The FC reagent will be added to the fatty acid solution, followed by sodium carbonate.
- After incubation, the absorbance will be measured at 765 nm.
- The reducing power will be expressed as gallic acid equivalents.

Reactive Oxygen Species (ROS) Scavenging Activity:

- The ability of the fatty acids to scavenge hydrogen peroxide will be assessed by mixing the fatty acid solution with hydrogen peroxide in a phosphate buffer.
- The absorbance will be measured at 230 nm after 10 minutes.
- The percentage of ROS scavenging will be calculated.

4. Enzyme Inhibitory Activity

Materials:

- Isolated fatty acids
- α-amylase, α-glucosidase, and lipase enzymes
- Starch, p-nitrophenyl-α-D-glucopyranoside, p-nitrophenyl butyrate
- Phosphate buffer

Methods:

- The enzyme inhibitory activity will be assessed by incubating the fatty acids with specific enzymes (α-amylase, α-glucosidase, and lipase) and their substrates (starch, p-nitrophenyl-α-D-glucopyranoside, p-nitrophenyl butyrate).

- The reaction mixture will be incubated at 37°C, and the absorbance will be measured at specific wavelengths (540 nm for α-amylase, 405 nm for α-glucosidase and lipase).
- The percentage inhibition will be calculated by comparing the absorbance of the control and test samples.

5. Anti-diabetic Activity

Materials & Methods:

- Isolated fatty acids
- Streptozotocin-induced diabetic rats
- Oral glucose tolerance test (OGTT) kits
- Blood glucose meter

- The anti-diabetic potential of the fatty acids will be tested using streptozotocin-induced diabetic rats.
- The rats will be divided into control and test groups, with the test groups receiving different concentrations of fatty acids.
- Oral glucose tolerance tests will be performed by administering glucose after fasting, followed by measuring blood glucose levels at intervals.
- The reduction in blood glucose levels in the test groups will be compared to the control group to evaluate the anti-diabetic effect.

6. Evaluation of Health-Promoting Effects

Materials:

- Isolated fatty acids
- Inflammatory markers (e.g., TNF-α, IL-6)
- Cardiovascular markers (e.g., cholesterol, triglycerides)
- Immune system markers (e.g., immunoglobulins)
- Cell culture models (e.g., macrophages, endothelial cells)
- Animal models (if applicable)
- ELISA kits, RT-PCR, Western blotting

Methods:

- The potential health-promoting effects of fatty acids will be studied by assessing their impact on inflammatory, cardiovascular, and immune system markers.
- Inflammatory responses will be evaluated using cell culture models treated with fatty acids, followed by the measurement of inflammatory cytokines (e.g., TNF-α, IL-6) using ELISA.
- The impact on cardiovascular health will be studied by measuring cholesterol, triglycerides, and other lipid profiles in animal models or cell cultures.
- The immune-boosting effects will be assessed by evaluating the levels of immunoglobulins and the expression of immune-related genes using RT-PCR and Western blotting.
- The findings will be correlated to assess the overall health-promoting potential of the fatty acids in reducing the risk of heart disease, reducing inflammation, and strengthening the immune system.

7. Data Analysis

Methods:

- The collected data will be analyzed using statistical software such as SPSS or GraphPad Prism.
- Descriptive statistics will be used to summarize the data, including mean, standard deviation, and percentage.
- Inferential statistics, including ANOVA and t-tests, will be employed to compare the effects of different concentrations of fatty acids on antimicrobial, antioxidant, enzyme inhibitory, anti-diabetic, and health-promoting activities.
- Correlation and regression analyses will be conducted to determine the relationships between fatty acid concentrations and the observed biological effects.
- Results will be presented as mean ± standard deviation, and a p-value of <0.05 will be considered statistically significant.

8. Ethics

Ethical Considerations:

- If animal models are used, the research will adhere to the ethical guidelines set forth by the University Ethics Committee (UEC).
- Approval will be sought from the UEC before the commencement of any experiments involving animals.
- All animal handling procedures will be conducted in compliance with the guidelines for the care and use of laboratory animals, ensuring minimal distress and pain.
- If human cell lines or tissues are used, approval will be obtained from the University Ethics Committee (UEC).
- Informed consent will be obtained from donors (if applicable), and confidentiality of donor information will be maintained.
- The research will ensure that all ethical guidelines, including those related to the humane treatment of animals and the ethical use of human tissues, are strictly followed throughout the study.

Expected Results

Potential Outcomes

Isolation and Characterization of Fatty Acids:

- The study is expected to successfully isolate a range of fatty acids from *Vicia faba* seeds, including saturated, monounsaturated, and polyunsaturated fatty acids.
- The Gas Chromatography-Mass Spectrometry (GC-MS) analysis will likely identify key fatty acids such as linoleic acid, oleic acid, and palmitic acid, among others.

Antimicrobial Activity:

- The isolated fatty acids are expected to exhibit significant antimicrobial activity against various bacterial strains, particularly gram-positive bacteria like *Staphylococcus aureus*.
- Zones of inhibition will likely vary depending on the concentration of fatty acids, with higher concentrations exhibiting stronger antimicrobial effects.

Antioxidant Capacity:

- The fatty acids are anticipated to show potent antioxidant activity, as evidenced by substantial scavenging of DPPH and ABTS radicals, along with high reducing power in the Folin-Ciocalteu assay.
- The fatty acids may also demonstrate effective ROS scavenging activity, indicating their potential to neutralize reactive oxygen species and reduce oxidative stress.

Enzyme Inhibitory Activity for Diabetes Management:

- The fatty acids are expected to inhibit the activity of key enzymes such as α-amylase, α-glucosidase, and protease, suggesting their potential in managing hyperglycemia by slowing down carbohydrate digestion and absorption.
- The degree of enzyme inhibition will likely correlate with the fatty acid structure, with certain fatty acids showing stronger inhibitory effects.

Health-Promoting Effects:

- The fatty acids are anticipated to exhibit anti-inflammatory properties by reducing the levels of pro-inflammatory cytokines such as TNF-α and IL-6 in cell culture models.
- They may also improve cardiovascular health by reducing lipid profiles, such as cholesterol and triglycerides, in animal models or cell cultures.
- Furthermore, the fatty acids are expected to boost the immune system by increasing immunoglobulin levels and enhancing the expression of immune-related genes.

Significance of Results

Contribution to Nutritional Science:

- The results will contribute to the understanding of the nutritional and therapeutic potential of *Vicia faba* seeds, positioning them as a valuable source of bioactive fatty acids with health benefits.
- The identification and characterization of specific fatty acids with antimicrobial, antioxidant, and enzyme inhibitory properties could lead to the development of functional foods or nutraceuticals aimed at improving human health.

Advancements in Disease Management:

- The study's findings on the enzyme inhibitory activity of fatty acids could have significant implications for the management of diabetes, offering natural alternatives to synthetic enzyme inhibitors.
- The potential anti-inflammatory and cardiovascular benefits of these fatty acids may provide new insights into preventing and managing chronic diseases such as heart disease and inflammatory disorders.

Innovation in Antimicrobial Therapies:

- The discovery of fatty acids with strong antimicrobial properties could lead to the development of new, plant-based antimicrobial agents, addressing the growing concern of antibiotic resistance.
- These findings could be particularly valuable in creating natural preservatives for food and pharmaceutical industries.

Impact on Public Health:

- The health-promoting properties of *Vicia faba* fatty acids, such as reducing inflammation and strengthening the immune system, may have broad public health implications, especially in the prevention of lifestyle-related diseases.
- The results could support dietary recommendations that encourage the consumption of *Vicia faba* as part of a balanced diet to promote overall health and well-being.

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